SYNOPSIS OF RESEARCH PROJECT

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8.	**Title of Research Project**	**:**	**STUDIES ON MORPHOLOGICAL DIVERSITY, REPRODUCTIVE BIOLOGY AND INTERSPECIFIC HYBRIDIZATION IN WILD *SOLANUM* SPECIES FOR BRINJAL (*Solanum melongena* L.) IMPROVEMENT**

9. Introduction

Brinjal or eggplant (*Solanum melongena* L.) is one of the most important vegetable crops belonging to the family Solanaceae and is widely cultivated in tropical and subtropical regions of the world. It occupies a prominent position among vegetable crops due to its high nutritional value, culinary versatility and economic importance. Brinjal is consumed in a wide variety of dishes and is an essential component of diets in many Asian and African countries. Globally, eggplant production is estimated to be about 59 million tonnes annually, with China and India contributing the majority of the production. China accounts for nearly 65% of global eggplant production, while India is the second largest producer in the world and contributes a significant share to global output (World Population Review, 2026). The eggplant is a rich source of vitamins, minerals, dietary fiber and bioactive compounds such as phenolics and flavonoids, which contribute to its antioxidant properties. Due to its adaptability to diverse climatic

conditions and year-round cultivation potential, it has become an important vegetable crop for both commercial farming and household consumption.

India is considered one of the major centres of diversity for brinjal and its related species. Major brinjal producing states in India include West Bengal, Odisha, Gujarat, Madhya Pradesh, Bihar and Chhattisgarh, with West Bengal alone contributing about 24% of the total national production (DesiKheti, 2025). The country possesses a rich diversity of cultivated varieties and wild relatives belonging to the genus *Solanum*.

The genus *Solanum* is one of the largest genera among flowering plants, comprising more than 1,500 species distributed throughout the world. Many economically important crops such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and brinjal (*Solanum melongena*) belong to this genus. Within the *Solanum* genus, the brinjal complex includes several cultivated and wild species that share genetic relationships and evolutionary history. These species exhibit significant diversity in terms of morphology, growth habit, fruit characteristics and ecological adaptation.

Wild relatives of brinjal belonging to the genus *Solanum* are widely distributed in tropical and subtropical regions of the world and occur naturally in a variety of habitats including forests, wastelands, roadsides and agricultural field margins. In India, several wild species such as *Solanum incanum*, *Solanum nigrum*, *Solanum torvum*, *Solanum diphyllum, Solanum aethiopicum* and *Solanum sisymbrifolium* are commonly reported and contribute significantly to the genetic diversity of the eggplant complex. *Solanum incanum* L., considered one of the wild progenitors of cultivated brinjal, is widely distributed in dry and semi-arid regions of India including Rajasthan, Gujarat, Madhya Pradesh and parts of southern India, where it grows as a spiny shrub in open fields, wastelands and scrub forests (Lester and Hasan, 1991; Vorontsova and Knapp, 2016). Another widely distributed species, *Solanum nigrum* L. (black nightshade), occurs throughout India in cultivated lands, roadsides, gardens and disturbed habitats, particularly in temperate and tropical regions (Edmonds and Chweya, 1997).

Similarly, *Solanum torvum* Sw., commonly known as turkey berry, is distributed in tropical and subtropical regions and occurs in northeastern and southern parts of India including Assam, Arunachal Pradesh, Kerala and Tamil Nadu, where it grows in forest margins and wastelands (Knapp *et al.*, 2013). *Solanum aethiopicum* L., commonly referred to as Ethiopian eggplant or scarlet eggplant, though native to Africa, is cultivated and naturalized in parts of northeastern India such as Manipur, Mizoram and

Nagaland, where it is used as a traditional vegetable crop (Schippers, 2000). Another wild species, *Solanum sisymbrifolium* Lam., commonly known as litchi tomato or sticky nightshade, has been reported from northern and eastern parts of India including Uttar Pradesh, Jharkhand and Tripura, where it grows in disturbed habitats such as roadsides and agricultural fields (Kumar *et al.*, 2022). *Solanum diphyllum* is commonly found growing in gardens, roadsides, wastelands and semi-natural habitats, particularly in southern and northeastern states such as Karnataka, Tamil Nadu, Kerala, Assam and West Bengal. The plant adapts well to warm climatic conditions and disturbed habitats.

The wide distribution of wild *Solanum* species across diverse agro ecological regions of India reflects their adaptability and rich genetic variability. These species serve as valuable genetic resources for brinjal improvement, particularly for enhancing resistance to pests, diseases and environmental stresses. Many wild relatives possess inherent resistance to important biotic stresses and have been effectively utilized in breeding programmes. For instance, *Solanum torvum* is widely used as a rootstock due to its strong resistance to bacterial wilt and nematodes, resulting in improved plant vigor and productivity in cultivated brinjal (Gisbert *et al.*, 2011). Similarly, *Solanum sisymbrifolium* acts as a trap crop for root-knot nematodes by inducing egg hatching but preventing their development, thereby reducing nematode populations in the soil. Other wild species such as *S. diphyllum* also represent important sources of genetic variability and can contribute novel genes for stress tolerance and pest resistance in cultivated eggplant (*Solanum melongena*).

Despite its economic importance, the productivity of brinjal is severely affected by several biotic and abiotic stresses. Among the biotic stresses, insect pests and diseases are major limiting factors affecting yield and fruit quality. The shoot and fruit borer (*Leucinodes orbonalis*) is considered one of the most destructive insect pests of brinjal and can cause yield losses. In addition to insect pests, several diseases such as bacterial wilt (*Ralstonia solanacearum*), root-knot nematodes (*Meloidogyne spp*.) and viral diseases also pose serious challenges to brinjal cultivation. Although chemical pesticides are commonly used to manage these problems, excessive reliance on chemicals increases production costs and poses threat to environment and human health. Therefore, the development of pest and disease resistant cultivars through plant breeding remains one of the most effective and sustainable strategies for crop improvement.

One of the major challenges in modern agriculture is the limited genetic diversity present in many cultivated crops. Continuous selection and breeding for specific traits have often resulted in a narrow genetic base, making crops more vulnerable to pests, diseases and environmental stresses. Wild relatives of crop plants provide an opportunity to broaden the genetic base by introducing novel genes and traits into cultivated varieties. Through conventional breeding techniques as well as modern biotechnological approaches, beneficial traits from wild species can be transferred to cultivated crops to enhance their productivity and resilience.

The conservation and utilization of wild plant species have become increasingly important in the context of global food security and climate change. Habitat destruction, urbanization and agricultural expansion pose significant threats to the survival of many wild species. Therefore, systematic efforts are required to collect, characterize and conserve wild germplasm before it is lost. Germplasm conservation programs play a vital role in preserving genetic diversity and ensuring the availability of valuable genetic resources for future research and crop improvement.

Morphological characterization is an essential step in the study of plant genetic resources. It involves the evaluation of observable plant traits such as plant height, leaf shape, flower characteristics, fruit size, fruit colour and seed attributes. Such studies help in identifying the extent of variability present among different species or accessions. In the case of wild brinjal species, morphological characterization can provide valuable insights into their diversity, adaptation and potential use in breeding programs. In addition to morphological studies, understanding seed biology and germplasm preservation is also important for maintaining wild plant resources. Proper methods of seed extraction, drying, storage and viability testing are essential to ensure the long-term conservation of genetic material. Seeds of wild brinjal species often exhibit varying storage behaviour and dormancy patterns, which need to be studied carefully to develop effective conservation strategies.

Considering the increasing importance of wild relatives in crop improvement and biodiversity conservation, the study of wild brinjal species such as *Solanum aethiopicum*, *Solanum torvum, Solanum diphyllum* and *Solanum sisymbrifolium* is highly relevant. These species possess unique morphological characteristics and valuable traits that can help in the development of improved brinjal varieties. Understanding their biology, variability and potential applications can provide useful information for plant breeders, geneticists and conservationists.

Therefore, systematic research on these wild species is necessary to explore their genetic potential and to utilize them effectively in breeding programs. The collection, characterization and evaluation of wild brinjal germplasm can help identify desirable traits that may be incorporated into cultivated brinjal through appropriate breeding strategies. Such efforts will not only enhance crop productivity but also contribute to sustainable agricultural practices and long-term food security.

10. Practical Utility of the Research Project

Despite the availability of diverse wild *Solanum* species, their effective utilization in breeding programmes remains limited due to limited understanding of their reproductive behaviour, crossability relationships and hybrid performance. Evaluation of pollen viability and fertility is a fundamental prerequisite for identifying suitable parental lines, as it directly influences the success of hybridization. Variations in pollen viability among wild species can significantly affect fertilization efficiency and subsequent hybrid development, making its assessment essential for selecting compatible genotypes.

Following this, understanding crossability relationships among selected *Solanum* species is crucial for determining the success of interspecific hybridization. The extent of fruit set, seed set and hybrid survival varies depending on genetic compatibility and direction of crossing, indicating the presence of both pre- and post-fertilization barriers. Several studies have reported that while some crosses result in fruit formation, the seeds are often few, non-viable or exhibit poor germination due to post-zygotic barriers such as embryo abortion and improper endosperm development (Plazas *et al.*, 2016; Afful *et al.*, 2020).

In cases where viable hybrids are obtained, characterization of F_1 hybrids is essential to confirm hybridity and to assess the expression of morphological traits. Interspecific hybrids often exhibit intermediate characteristics between parents and may show variability in growth, fertility and other agronomic traits, which are important for further utilization in breeding programmes.

However, in wide crosses involving distantly related wild species, the success of hybrid recovery is often severely restricted due to strong post-zygotic barriers. To overcome these limitations, embryo rescue has emerged as an effective technique, involving the *in vitro* culture of immature embryos or ovules at early developmental stages to bypass seed failure. This approach has been successfully employed to recover hybrids in *Solanum* crosses where conventional methods fail (Mamedov *et al.*, 2013;

Kumchai *et al.*, 2013). In eggplant, embryo rescue and related *in vitro* techniques have facilitated the development of interspecific hybrids involving *S. melongena* and wild relatives such as *S. torvum*, *S. sisymbriifolium* and *S. incanum*, which are otherwise difficult to obtain due to strong incompatibility barriers (Rakha *et al.*, 2020; Kumchai *et al.*, 2013).

In addition to hybridization barriers, susceptibility to major biotic stresses such as insect pests and diseases remains a major limitation in brinjal production, leading to significant yield losses. Wild relatives of *Solanum* are important sources of resistance to several biotic stresses, including shoot and fruit borer, bacterial wilt and root knot nematodes. The utilization of these wild species through interspecific hybridization provides a sustainable approach for improving resistance in cultivated brinjal. Therefore, evaluation of wild *Solanum* species and their interspecific hybrids for resistance to major biotic stresses is essential for identifying suitable donor parents and developing improved brinjal genotypes.

Therefore, integration of conventional hybridization with embryo rescue techniques is essential for improving the efficiency of distant hybridization and enabling the utilization of wild genetic resources in brinjal improvement. In this context, the present study aims to systematically evaluate pollen viability and fertility, assess crossability relationships and hybridization success, characterize the resulting F_1 hybrids and apply embryo rescue techniques to overcome post-zygotic barriers in selected wild *Solanum* species. The findings are expected to facilitate the identification of compatible cross combinations, enhance hybrid recovery and contribute to the development of pre-breeding materials for broadening the genetic base of brinjal.

11. Objectives

The present investigation will be carried out with the following objectives:

1. To evaluate pollen viability and pollen fertility in wild species of genus *Solanum*
2. To study crossability relationships and distant hybridization success among wild *Solanum* species through all possible interspecific cross combinations
3. To utilize embryo rescue techniques to overcome post-zygotic barriers in interspecific hybridization of wild *Solanum* species
4. To evaluate and characterize the morphological traits of parental lines and F_1 hybrids obtained from successful crosses
5. To evaluate wild *Solanum* species and their interspecific hybrids for resistance to major biotic stresses of brinjal

12. Review of Literature

Wild relatives of brinjal within the genus *Solanum* have gained considerable importance in recent years due to their potential role in improving cultivated eggplant through the transfer of adaptive and resistance traits. These species exhibit wide variation in morphological, genetic and reproductive characteristics, which are largely absent in cultivated *S. melongena*. Interspecific hybridization studies involving wild taxa such as *Solanum torvum* have demonstrated their potential as sources of resistance and genetic variability, but the resulting hybrids often exhibit reduced fertility and reproductive instability, particularly in terms of pollen viability (Kumchai *et al.,* 2013). Similarly, cytogenetic studies involving crosses between *S. incanum* and cultivated eggplant have shown that although chromosome pairing may occur normally, partial pollen sterility is often observed in hybrid progenies, indicating genomic imbalance and incompatibility (Anis *et al.,* 1994). Investigations on less explored species such as *S. diphyllum* further reveal that basic information on meiotic behaviour and reproductive efficiency is still limited, with only a few studies addressing these aspects (Verma *et al.,* 2016).

Despite these advancements, most available studies are restricted to individual species or specific cross combinations and there is a lack of systematic and comparative evaluation of reproductive traits across multiple wild *Solanum* species. In particular, the relationship between pollen fertility, crossability and hybrid success has not been comprehensively explored under uniform experimental conditions. This highlights a critical gap in understanding the reproductive behaviour of wild brinjal species, which is essential for their effective utilization in breeding programmes.

The available literature relevant to the objectives of the present study has been systematically reviewed and is presented under the following heads:

12.1 Pollen viability and reproductive biology

França *et al.* (2009) evaluated pollen viability in eggplant using different methodologies including *in vitro* and in vivo pollen germination tests as well as tetrazolium staining. The study reported that *in vitro* germination showed very low pollen viability, whereas in vivo germination on the stigma provided significantly higher viability, indicating its suitability for accurate assessment. In contrast, tetrazolium staining was found to be less effective due to poor staining response of pollen grains.

Oyelana and Ugborogho (2009) studied pollen viability in interspecific hybrids derived from crosses among *Solanum melongena*, *S. macrocarpon* and *S. aethiopicum*. The results showed that pollen viability was significantly reduced in the hybrids compared to the parental species, declining from 71-97.3 per cent in the parents to 48.8-56.8 per cent in the F_1 generation and further to 38.2-48.6 per cent in the F_2 generation. The reduction in pollen fertility indicated the presence of genomic incompatibility and meiotic irregularities in interspecific hybrids, which adversely affected reproductive efficiency.

Abdullateef *et al.* (2012) performed an experiment on pollen viability in different *Solanum* species including *S. melongena* and *S. gillo* along with their inter-varietal and interspecific hybrids. The results indicated that pollen viability in parental lines ranged from 82.3 to 83.3 per cent in *S. melongena* accessions and 42.5-67.0 per cent in *S. gillo* types. Among the hybrids, inter-varietal crosses such as SM-1 × SM-2 and SG-4 × SG-5 recorded higher pollen viability (65.9–76.0%), whereas interspecific crosses showed lower fertility ranging from 21.2 to 63.7 per cent. The lowest pollen viability was observed in interspecific combinations like SM-1 × SG-5, while higher values in certain crosses indicated better compatibility. Significant differences among genotypes and hybrids suggest that pollen viability varies depending on genetic background and type of cross.

Kumchai *et al.* (2013) developed interspecific hybrids between cultivated eggplant (*Solanum melongena* L.) and its wild relative *S. torvum* through cross-hybridization followed by embryo rescue. The parental lines, including *S. melongena* cultivars and *S. torvum*, exhibited very high pollen viability, approaching nearly 100 per cent with uniform and well-developed pollen grains. In contrast, the interspecific hybrid progenies showed a drastic reduction in pollen viability, with viable pollen ranging from 0 to 9.8 per cent, while a large proportion of pollen grains were either rudimentary (4.5-96.2%) or empty (1.3-95.5%). Some hybrid combinations exhibited almost complete pollen sterility, indicating severe reproductive instability. This reduction in pollen fertility was attributed to meiotic abnormalities and genetic incompatibility between the parental species.

Ghani *et al.* (2020) evaluated pollen viability in both parental lines and their interspecific hybrids between *Solanum melongena* and *S. incanum* using acetocarmine staining. The results revealed that the cultivated parent showed high pollen fertility, whereas some hybrids exhibited reduced pollen viability, indicating partial sterility due

to genetic incompatibility. However, certain hybrid combinations maintained moderate to high pollen fertility, suggesting their potential use in further breeding programmes. The study highlights that pollen viability in interspecific hybrids is highly variable and largely influenced by the genetic relationship between parental species.

However, most studies are limited to specific species combinations and do not provide a comparative evaluation of pollen viability across multiple wild *Solanum* species under uniform conditions.

12.2 Crossability and distant hybridisation

Boyacı (2020) investigated interspecific hybridization between *Solanum melongena* and several wild relatives to enhance genetic diversity and develop introgression lines. The study revealed that successful hybridization was limited, as F_1 hybrids were obtained only in a few cross combinations among the nine wild species tested, indicating the presence of strong reproductive barriers. Crossability varied depending on the parental combinations, with species such as *S. incanum*, *S. macrocarpon* and *S. aethiopicum* showing relatively better compatibility. However, several crosses failed due to sexual incompatibility and some hybrids exhibited sterility or poor seed germination. The results further highlighted that even when hybrids were produced, their advancement to later generations was restricted due to recombination effects and reduced fertility. The study emphasized that despite these limitations, wild relatives possess significant potential for broadening the genetic base of eggplant and improving resistance traits, although their practical utilization in breeding programs remains limited due to crossability barriers.

Ghani *et al.* (2020) conducted interspecific and intraspecific hybridization in eggplant involving cultivated genotypes of *Solanum melongena* and the wild species *S. incanum* with the aim of transferring useful traits for crop improvement. A large number of reciprocal crosses were attempted, but only a few combinations resulted in successful hybrid formation, indicating the presence of strong crossability barriers. The success of hybridization was found to depend on the direction of crossing, as fruit set was obtained only when *S. incanum* was used as a pollen parent, while the reciprocal crosses failed. The interspecific hybrids exhibited intermediate morphological characteristics and showed improvement in certain traits such as fruit yield and vegetative growth. The study clearly demonstrated that although wild species possess valuable traits, their utilization through distant hybridization is often constrained by reproductive barriers and genotype-dependent compatibility.

Rakha *et al.* (2020) developed interspecific hybrids between cultivated eggplant (*Solanum melongena*) and several wild relatives including *S. incanum, S. insanum, S. anguivi* and *S. sisymbriifolium* to evaluate crossability and their potential in breeding programs. The study reported that successful hybridization was highly dependent on the species involved and the direction of the cross. Higher fruit set was observed in crosses involving *S. incanum* in both directions, indicating better compatibility, whereas no successful crosses were obtained with *S. sisymbriifolium*, confirming its distant relationship with cultivated eggplant. Seed set, germination and hybrid recovery also varied significantly among species combinations. In certain crosses, particularly with *S. incanum*, embryo rescue techniques were required to obtain viable hybrids, highlighting the presence of strong post-fertilization barriers. The results clearly demonstrated that although interspecific hybridization is feasible in eggplant, reproductive barriers such as low seed development and hybrid incompatibility restrict the success of distant crosses.

Nasiruddin *et al.* (2021) conducted interspecific hybridization between cultivated *Solanum melongena* and its wild relatives *S. insanum* and *S. incanum* with the objective of introgressing resistance against fruit and shoot borer. The study demonstrated that successful hybridization could be achieved using cultivated brinjal as the female parent and wild species as pollen donors. The degree of crossability varied among different parental combinations, as reflected by differences in fruit set, seed production and germination. Higher hybridization success was observed in crosses involving *S. insanum* compared to *S. incanum*. The hybridity of F_1 plants was confirmed using RAPD markers, indicating effective gene transfer from wild to cultivated species. These findings highlight the potential of wild relatives in distant hybridization programmes for broadening the genetic base and improving resistance in brinjal.

Ranaweera *et al.* (2021) evaluated interspecific hybrids developed between cultivated eggplant (*Solanum melongena*) and wild relatives belonging to different gene pools including *S. incanum, S. insanum, S. anguivi, S. dasyphyllum* and *S. torvum*. The study highlighted that the success of interspecific hybridization in eggplant largely depends on the genetic relationship between species, where primary gene pool species such as *S. incanum* and *S. insanum* exhibited better compatibility and produced viable hybrids, while distant species from secondary and tertiary gene pools showed reduced crossability and fertility. The hybrids generally displayed morphological traits closer to the wild parents, indicating the dominance of wild alleles. Furthermore, the study

emphasized that although hybridization across gene pools is possible, the resulting hybrids often express undesirable traits such as excessive prickliness and small fruit size, requiring further selection and backcrossing. The findings also suggested that these interspecific hybrids serve as valuable pre-breeding material for introgression of desirable traits, despite limitations imposed by reproductive barriers.

Karmakar and Singh (2023) investigated interspecific hybridization among cultivated brinjal (*Solanum melongena*) and its wild relatives including *S. aethiopicum, S. gilo* and *S. khasianum* to evaluate crossability and hybrid performance. The study revealed that most interspecific crosses were successful, although the degree of crossability varied depending on the species combination and direction of crossing. Crosses involving *S. gilo* and *S. aethiopicum* showed good compatibility with cultivated brinjal, producing viable seeds and hybrids, whereas crosses involving *S. khasianum* as a female parent failed due to incompatibility barriers. In several cross combinations, parthenocarpic fruit development was observed, indicating incomplete fertilization. The study also reported reduced pollen fertility in F_1 hybrids compared to their parents, suggesting the presence of genetic incompatibility. Overall, the results demonstrated that while interspecific hybridization in eggplant is feasible, reproductive barriers such as poor seed germination, embryo degeneration and reduced fertility limit the efficiency of distant hybridization.

Despite extensive studies, the combined relationship between crossability, reproductive barriers and hybrid performance across diverse wild species remains inadequately explored.

12.3 Morphological characterization and diversity in wild *Solanum* and interspecific hybrids

Sakhanokho *et al.* (2014) evaluated intraspecific variability in *Solanum aethiopicum* using morphological and cytogenetic approaches across multiple accessions. The study revealed considerable variation among accessions, particularly in flower and fruit-related traits, indicating a high level of phenotypic diversity within the species. Cluster analysis grouped the accessions into distinct categories, demonstrating the presence of genetically diverse groups within *S. aethiopicum*. Such variability suggests that this species possesses significant potential as a genetic resource for breeding programs, especially for the selection of desirable traits and utilization in interspecific hybridization.

Mutegi *et al.* (2015) studied wild and weedy eggplant (*Solanum insanum*) populations from southern India to understand the extent of variability present in natural populations. The study revealed considerable variation among wild populations, which was influenced by geographical distribution. Distinct differences were observed among populations, indicating a high level of diversity within the species. The findings suggest that wild brinjal populations possess substantial variability, which can be effectively utilized for characterization and selection in crop improvement programmes.

Dhatt *et al.* (2017) carried out morphological characterization of eggplant along with its related wild species using a wide range of qualitative and quantitative traits. The study revealed significant variation among germplasm for traits such as plant height, leaf dimensions, fruit length, fruit diameter, fruit weight and number of primary branches. The clustering analysis based on morphological traits grouped the germplasm into distinct clusters, indicating substantial genetic diversity. The study also highlighted that wild species such as *Solanum incanum* and *S. aethiopicum* were genetically distinct from cultivated types and can serve as valuable genetic resources for broadening the genetic base and improving agronomic traits in eggplant breeding programmes.

Sanap *et al.* (2021) developed interspecific hybrids between cultivated *Solanum melongena* and the wild species *S. insanum* and evaluated them for morphological characterization. The study reported that the F_1 hybrids exhibited intermediate plant morphology between the parents. Morphological traits such as plant growth habit, plant height, leaf characters, spine presence, inflorescence type, stigma position and fruit characteristics were assessed and most traits showed intermediate expression in the hybrids, reflecting the contribution of both parental species.

Saavedra *et al.* (2024) evaluated introgressed eggplant lines developed using the wild species *Solanum incanum* under water stress conditions. The study observed variation in morphological traits such as plant growth, leaf characteristics and biomass among the introgressed lines and their parents. The cultivated parent generally showed higher growth, whereas lines with wild genomic background exhibited reduced growth but better adaptation under stress. The findings indicate that incorporation of wild genetic material influences plant morphology and performance, highlighting the role of *S. incanum* in developing stress-tolerant and diverse breeding lines.

12.4 Application of embryo rescue technique to avoid post-zygotic barrier in interspecific hybridisation

Mamedov *et al.* (2013) reported that interspecific hybridization in *Solanum* is often limited by strong post-zygotic barriers, particularly embryo abortion and failure of seed development, which restrict the recovery of viable hybrids through conventional methods. The study highlighted the use of embryo rescue techniques as an effective approach to overcome these limitations wherein immature embryos obtained from interspecific crosses are excised and cultured *in vitro* under controlled conditions to support their development into plantlets. This method allows the recovery of hybrids that would otherwise fail to survive due to incompatibility between parental species. The findings emphasize that embryo rescue is a critical tool in *Solanum* breeding programs for facilitating gene transfer from wild species into cultivated backgrounds by overcoming post-zygotic reproductive barriers.

Plazas *et al.* (2016) conducted extensive interspecific hybridization studies between cultivated eggplant (*Solanum melongena*) and a wide range of wild relatives belonging to different gene pools to evaluate crossability and hybrid recovery. A large number of crosses were attempted and parameters such as fruit set, seed development and germination were recorded. The study found that species from the primary and secondary gene pools showed relatively higher compatibility, producing viable seeds and hybrids, whereas crosses with tertiary gene pool species like *S. torvum* and *S. sisymbriifolium* exhibited strong post-zygotic barriers, including poor seed development and parthenocarpic fruits. In such cases, embryo rescue techniques were necessary to recover viable hybrids. The findings highlight that the success of interspecific hybridization in *Solanum* largely depends on genetic distance between species and emphasize the importance of embryo rescue for overcoming post-zygotic barriers in distant crosses.

Afful *et al.* (2020) conducted a study on interspecific hybridization in eggplant involving crosses between cultivated *Solanum melongena* and wild species such as *S. torvum* and *S. anguivi* to improve hybrid recovery through *in vitro* techniques. The authors performed controlled crosses and observed that although hybrid fruits were formed, they often contained few or non-viable seeds due to strong post-zygotic barriers such as embryo abortion and poor seed development. To address this, seeds and excised embryos obtained from hybrid fruits were cultured on Murashige and Skoog (MS) medium supplemented with plant growth regulators like IAA and BAP. The study found

that *in vitro* seed culture improved germination and seedling survival, whereas excised embryos showed limited development under certain conditions. These results indicate that conventional methods alone are inadequate for successful hybrid recovery in some *Solanum* crosses and the application of *in vitro* techniques, including embryo rescue, is essential to overcome post-zygotic barriers and enhance hybridization success.

Rakha *et al.* (2020) developed interspecific hybrids between cultivated eggplant (*Solanum melongena*) and selected wild relatives with the objective of evaluating crossability and hybrid development. Controlled crosses were performed using different wild species in both direct and reciprocal directions and parameters such as fruit set, seed production and hybrid survival were assessed. The study revealed that hybridization success varied widely depending on the species and direction of crossing, indicating the presence of both pre- and post-zygotic barriers. In particular, crosses involving *S. incanum* required the use of embryo rescue techniques to obtain viable hybrid plants, while some other combinations produced seeds only when the wild species was used as the female parent. These findings demonstrate that interspecific hybridization in *Solanum* is highly dependent on genetic compatibility and cross direction and highlight the essential role of embryo rescue in overcoming post-zygotic barriers and ensuring successful hybrid recovery.

Kaur *et al.* (2023) carried out interspecific hybridization between cultivated eggplant (*Solanum melongena*) and the wild species *S. sisymbriifolium* to study cross compatibility and improve hybrid recovery using embryo rescue techniques. The researchers performed controlled crosses and observed very low success in terms of fruit set and seed development due to strong reproductive barriers. To overcome this, immature embryos were excised from developing fruits (around 25 days after pollination) and cultured *in vitro* on half-strength MS medium supplemented with growth regulators. This approach resulted in successful embryo germination and the development of viable plantlets, although the hybrids exhibited partial fertility and morphological variation. The study clearly demonstrated that embryo rescue is an effective strategy to overcome post-zygotic barriers in difficult interspecific crosses of *Solanum*, enabling the recovery of hybrids that are otherwise not obtainable through conventional methods.

12.5 Biotic stress resistance in wild *solanum* species and interspecific hybrids

Behera and Singh (2002) studied resistance to shoot and fruit borer (*Leucinodes orbonalis*) in different *Solanum* species including cultivated varieties, wild relatives

and their interspecific hybrids under field conditions. The results indicated that wild species such as *S. indicum*, *S. incanum* and *S. gilo* showed lower levels of infestation compared to cultivated types, demonstrating their inherent resistance. Among them, *S. indicum* exhibited almost complete immunity to fruit infestation. In the hybrids, crosses like Pusa Kranti × *S. indicum* recorded reduced shoot and fruit damage compared to susceptible parents, indicating successful transfer of resistance traits. The study clearly suggested that wild *Solanum* species can serve as effective sources of resistance and their utilization through interspecific hybridization can improve pest resistance in brinjal.

Pugalendhi *et al.* (2010) conducted an experiment to transfer resistance to shoot and fruit borer using *Solanum viarum* as a wild donor parent through interspecific hybridization in brinjal. The hybrids derived from *S. melongena* × *S. viarum* were advanced up to the F_9 generation with continuous selection for desirable traits. The results showed that selected progenies exhibited very low shoot and fruit infestation along with higher marketable yield compared to the susceptible parent. The study also indicated that resistance traits from wild species could be successfully introgressed into cultivated backgrounds through selection in later generations. These findings highlight the importance of wild relatives as valuable genetic resources for improving resistance to major insect pests.

Gousset *et al.* (2005) evaluated the resistance potential of *Solanum torvum*, a wild relative of brinjal, against major soil-borne diseases such as bacterial wilt and Fusarium wilt. The study reported that all tested accessions of *S. torvum* exhibited strong resistance or tolerance, whereas cultivated *S. melongena* lines were highly susceptible and showed severe wilting symptoms. The results further indicated that although wild species possess strong resistance, transferring these traits through interspecific hybridization is difficult due to incompatibility barriers such as sterility. However, the study emphasized that wild *Solanum* species are important sources of resistance genes and can be utilized in breeding programs using advanced techniques.

Neeraja *et al.* (2021) evaluated the resistance of *Solanum virginianum* accessions against brinjal shoot and fruit borer and compared them with cultivated varieties. The study revealed that wild accessions showed complete absence of shoot damage and significantly lower fruit infestation, whereas cultivated varieties exhibited higher susceptibility. The results further indicated that morphological and anatomical traits such as higher trichome density, thicker epidermis and presence of prickles

contributed to resistance against the pest. The study suggested that these structural traits act as defensive mechanisms and that *S. virginianum* can be effectively used as a donor parent in resistance breeding programs.

Boncukçu *et al.* (2023) conducted a study to evaluate resistance levels of wild *Solanum* species and their interspecific hybrid lines against *Verticillium dahliae* and *Fusarium oxysporum*. The results indicated considerable variation in disease resistance among genotypes, with several wild species showing high resistance compared to cultivated types. The interspecific hybrids also exhibited improved resistance levels, suggesting successful transfer of resistance traits from wild parents. The study highlighted that incorporation of wild germplasm into breeding programs can significantly enhance resistance to major soil-borne diseases in brinjal.

Sivasankarreddy *et al.* (2025) carried out large scale screening of diverse *Solanum* species and eggplant accessions for resistance to bacterial wilt and fruit borer under field conditions across multiple seasons. The results showed significant variation among genotypes, with several wild species such as *S. torvum*, *S. aethiopicum* and *S. viarum* exhibiting high levels of resistance to bacterial wilt. Additionally, some accessions also showed resistance to fruit borer infestation. The study demonstrated that wild *Solanum* species possess valuable resistance traits against multiple biotic stresses and can be utilized for developing improved brinjal varieties through breeding programs.

13. Material and Methods

13.1	**Location**	:	Main Vegetable Research Station, JAU, Junagadh
13.2	**Year and season of experiment**		
	a) For development of crosses	:	2025-26
	b) For evaluation of experimental materials	:	2026-27
13.3	**Crossing pattern**	:	Full diallel mating design
13.4	**Experimental design**	:	Randomized Block Design (RBD)
13.5	**Number of crosses**		30 (including the reciprocals)
13.6	**Wild species of brinjal used**	:	Six wild species of brinjal will be used

Table 1. Details of wild brinjal species used in the hybridization programme

S.N.	Species	Salient Features	Source
1	*Solanum torvum*	• Vigorous growth habit • Small, clustered fruits • Presence of spines • Known for pest and disease resistance	Collected from the local sources in Junagadh
2	*Solanum incanum*	• Spiny plant type • Small round fruits • Drought tolerant • Source of resistance traits	Collected from farmer's field (Banaskantha) and roadside areas (Ahmedabad)
3	*Solanum aethiopicum*	• Bushy growth habit • Variable fruit shape and colour • Moderate resistance to pests • High genetic variability	Northeast region - household area of Manipur
4	*Solanum diphyllum*	• Small shrub type plant • Yellow to orange berries • Ornamental type • Less compatibility with cultivated brinjal	Local area (Sapur, Junagadh)
5	*Solanum nigrum*	• Herbaceous plant type • Small black berries • Medicinal importance • Distantly related species	Nursery of Junagadh Agricultural University, Junagadh
6	*Solanum sisymbriifolium*	• Highly spiny plant • Lobed leaves • Small fruits • Strong resistance to pests	Local area (Sapur, Junagadh)

***Other species of *Solanum viz*., *S. insanum*, *S. khasianum* (*violaceum*), *S. indicum*, *S. anguivi*, etc. may be utilized in the programme depending upon the availability of the germplasm.**

13.7 Observations to be recorded

To ensure accuracy, data will be collected from five randomly selected competitive plants per plot, excluding those on the borders. While flowering and picking data (first, last and total count) will be recorded on a per-plot basis, specific metrics for pollen viability and crossability will also be documented to evaluate the success of interspecific hybridization among wild brinjal species.

13.7.1 Seed germination (%)

This value indicates the proportion of seeds that sprout successfully under controlled conditions. A set number of seeds per cross will be sown in germination trays, with success defined by visible radicle emergence within a specific timeframe. The final percentage will be determined using the following formula

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

13.7.2 Days to first flowering

The number of days from transplanting to first flower opening will be recorded on a plot basis for each entry. The observation will be taken when the first flower is fully opened, indicating earliness of flowering.

13.7.3 Pollen viability (%)

Pollen viability will be assessed using 1% acetocarmine staining technique. Viable pollen grains will appear well stained, while non-viable grains remain unstained and counts will be taken under a microscope. Pollen viability will be expressed as the percentage of viable pollen grains out of the total observed.

$$\text{Pollen viability (\%)} = \frac{\text{Number of viable pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

13.7.4 Pollen size (µm)

Pollen size will be determined by measuring the diameter of pollen grains collected from freshly opened flowers using a compound microscope fitted with an ocular micrometer.

13.7.5 Pollen germination (%)

Pollen germination will be assessed using *in vitro* germination technique by placing pollen grains on a suitable nutrient medium containing sucrose and boric acid. The pollen grains that produce a pollen tube will be considered as germinated. The number of germinated and non-germinated pollen grains will be counted under a microscope.

$$\text{Pollen germination}(\%) = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

13.7.6 Number of crosses attempted

The total number of crosses attempted for each cross combination will be recorded by counting the number of flowers emasculated and pollinated. This observation will be maintained for each cross to assess the extent of hybridization efforts.

13.7.7 Number of successful crosses

The number of successful crosses will be recorded based on the number of pollinated flowers that develop into fruits. This observation indicates the success of hybridization and compatibility between parental lines.

13.7.8 Fruit set (%)

Fruit set percentage will be calculated based on the number of fruits formed out of total crosses attempted for each cross combination. The observation will be recorded after fruit development and fruit set will be expressed as percentage using the ratio of successful crosses to total crosses attempted.

$$\text{Fruit set}\ (\%) = \frac{\text{Number of fruits set}}{\text{Total number of crosses attempted}} \times 100$$

13.7.9 Days to embryo culture

The number of days from pollination to embryo excision and culture will be recorded. This observation will help determine the appropriate stage of embryo development for successful *in vitro* culture.

13.7.10 Number of embryos cultured

The total number of embryos excised and cultured from each successful cross will be recorded. This observation will indicate the efficiency of embryo recovery from interspecific crosses and the material available for *in vitro* culture

13.7.11 Embryo germination (%)

The number of embryos that successfully germinate under *in vitro* conditions will be recorded for each cross combination. Germination percentage will be calculated to assess the effectiveness of the culture medium and embryo rescue technique.

$$\text{Embryo germination}\ (\%) = \frac{\text{Number of embryos germinated}}{\text{Total number of embryos cultured}} \times 100$$

13.7.12 Shoot induction (%)

The number of embryos that develop shoots after germination will be recorded. This observation reflects the ability of rescued embryos to undergo further growth and differentiation under *in vitro* conditions.

$$\text{Shoot induction (\%)} = \frac{\text{Number of embryos producing shoots}}{\text{Total number of embryos germinated}} \times 100$$

13.7.13 Root induction (%)

The number of regenerated shoots that successfully produce roots will be recorded. This observation indicates the efficiency of rooting and successful plantlet formation.

$$\text{Root induction (\%)} = \frac{\text{Number of shoots producing roots}}{\text{Total number of shoots}} \times 100$$

13.7.14 Survival rate (%)

The number of plantlets that survive after transfer from *in vitro* conditions to greenhouse or field conditions will be recorded. This observation reflects the success of acclimatization and establishment of rescued plants.

$$\text{Embryo germination (\%)} = \frac{\text{Number of surviving plants}}{\text{Total number of plantlets transferred}} \times 100$$

13.7.15 Number of infested shoots per plant

The number of infested shoots per plant will be recorded by selecting five representative plants from each entry and counting all shoots showing drooping, wilting or dead heart symptoms. The observations will be recorded at regular intervals and the average number of infested shoots per plant will be calculated.

13.7.16 Shoot infestation (%)

Shoot infestation percentage represents the proportion of infested shoots in relation to the total number of shoots per plant. The total number of shoots (healthy and infested) and the number of infested shoots will be counted from selected plants and the percentage of shoot infestation will be calculated.

$$\text{Shoot infestation (\%)} = \frac{\text{Number of infested shoots}}{\text{Total number of shoots}} \times 100$$

13.7.17 Disease incidence (%)

Disease incidence represents the proportion of plants showing wilt disease symptoms in a given population. The number of infected plants and the total number of plants observed will be recorded and the disease incidence will be calculated.

$$\text{Disease incidence (\%)} = \frac{\text{Number of wilted plants}}{\text{Total number of plants per entry}} \times 100$$

13.7.18 Number of fruits per plant

The total number of fruits obtained from randomly selected plants in each entry will be recorded over all pickings. The average number of fruits per plant will be calculated based on total fruits harvested per plant.

13.7.19 Number of infested fruits per plant

The number of infested fruits per plant will be recorded by harvesting all fruits from selected plants at each picking and counting those showing damage. The average number of infested fruits per plant will be calculated.

13.7.20 Fruit infestation (%)

Fruit infestation percentage represents the extent of fruit damage in relation to the total number of fruits harvested. The number of infested and healthy fruits will be counted from selected plants and the percentage of fruit infestation will be calculated.

$$\text{Fruit infestation (\%)} = \frac{\text{Number of infested fruits}}{\text{Total number of fruits}} \times 100$$

13.7.21 Fruit length (cm)

Three fruits from randomly selected plants in each entry will be taken during peak harvesting period for recording fruit length. The length will be measured from the base of peduncle to the tip of the fruit in centimeters and the average fruit length will be calculated.

13.7.22 Fruit girth (cm)

Fruit girth will be measured from three selected fruits per plant taken from randomly selected plants in each entry during the peak harvesting period. The measurement will be taken at the middle portion of the fruit using vernier calipers and expressed in centimeters. The average fruit girth per plant will be calculated.

13.7.23 Average fruit weight (g)

Average fruit weight will be determined by weighing five randomly selected fruits from each plant during peak harvesting period using a digital balance. The weight will be recorded in grams and the average fruit weight per plant will be computed.

13.7.24 Fruit yield per plant (g)

Fruit yield per plant will be recorded by summing the total weight of fruits harvested from randomly selected plants in each entry over all pickings. The yield will

be measured in grams using a digital balance. The average fruit yield per plant will be calculated.

14. Statistical Analysis

The experimental data recorded for pollen viability, crossability parameters, morphological traits, embryo rescue efficiency and biotic stress parameters will be subjected to statistical analysis using appropriate statistical methods.

The data recorded for pollen studies, crossability parameters, embryo rescue efficiency and biotic stress parameters will be subjected to descriptive statistical analysis such as mean, variance and range to assess variation among wild *Solanum* species and interspecific hybrids using the formula given by Singh and Choudhary (1977).

14.1 Analysis of variance (ANOVA)

The data recorded for morphological traits and biotic stress parameters will be subjected to analysis of variance (ANOVA) to determine the significance of differences among wild *Solanum* species and interspecific hybrids following the method suggested by Panse and Sukhatme (1985).

14.2 Categorization of resistance to biotic stress

The extent of damage caused by shoot and fruit borer will be assessed based on shoot and fruit infestation.

Shoot damage will be calculated as the percentage of infested shoots by considering the ratio of terminal shoots showing drying or drooping symptoms to the total number of shoots per plant. Fruit damage will be estimated as percentage fruit infestation by counting the number of infested and non-infested fruits per plant.

Based on the mean percentage of shoot and fruit infestation throughout the growing period, the genotypes will be categorized into different resistance classes as per the grading scale suggested by Mishra *et al.* (1988), as presented in Table 2.

Table 2. Scoring for resistance to brinjal shoot and fruit borer (Mishra *et al.* 1988)

Grade	Score	Shoot and fruit damage (%)
Immune	1	0
Highly resistant	2	1–10
Moderately resistant	3	11–20
Tolerant	4	21–30
Susceptible	5	31–40
Highly susceptible	6	>40

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